**Case 19: Autoimmune lymphoproliferative disease (ALPS)**

**Summary:** ALPS is characterized by splenomegaly and lymphadenopathy from early childhood and often autoimmunity. Patients can develop autoimmune hemolytic anemia, neutropneia, thrombocytopenia, and hepatitis. Increased risk for developing lymphoma because B and T cells aren’t eliminated normally. Most patients are heterozygous for a dominant mutation in the FAS gene and their activated T cells don’t undergo Fas-mediated apoptosis in vitro (if one element of the trimer is mutant the trimer is ineffective and can’t deliver the signal to downstream elements of the pathway that ultimately cause cell death). Some patients will show impaired lympocyte apoptosis in vitro even if they don’t show any clinical symptoms (shows that there are environmental and/or genetic factors that have a role in full expression of ALPS phenotypes- variable expressivity).

Show elevated serum levels of FasL, IL10, and vitamin B12 (reliable biomarkers), along with increased DN T lymphocytes (these aren’t the same as normal DN thymocytes in T cell development). When B cells are activated they do not undergo normal Fas-mediated apoptosis (B cells usually express Fas) so serum concentrations of Igs increase (hypergammaglobulinemia), the number of B cells is increased (B cell lymphocytosis) and pathological autoantibody production occurs (may result because Fas-mediated killing is a mechanism for removing autoreactive B cells).

In some cases ALPS is due to somatic mutations of FAS that occur in an early lymphoid progenitor. Because of the impairment of apoptosis, the proportion of lymphocytes carrying the somatic mutations may increase over time and is particuarly high among DN T cells. In other patients, there are mutations for the genes encoding FasL or caspase 10. One case involved a GOF mutation in the NRAS gene that resulted in impaired induction of apoptosis in response to IL2 deprivation due to impaired induction of the pro-apoptotic molecule Bim which controls mitochondrial stability upon cytokine deprivation. Could have a missense mutation in caspase 8 at the site where it interacts with the Fas complex (might interfere with its function in Fas-induced apoptosis but not in other Fas-independent processes).

Similar lymphoproliferative disease in mice with lpr (no Fas) or gld (no FasL): progressive accumulation of DN T cells, antibodies against dnDNA.

TUNEL assay: performed in blood mononuclear cells, cells stimulated in vitro with phytohemagglutinin for 3 days, and growth of resulting T cell blasts continued for 3 weeks by addition of IL2, cultured then divided and half were exposed to antibody to Fas which mimics FasL, DNA in apoptotic cells is fragmented, label the fragments with terminal deoxynucleotidytransferase (TdT) which adds NTs to the ends of the fragments (biotin-labeled, usually dUTP), biotinylated DNA can be detected by using streptavidin, which binds to biotin, coupled to enzymes that convert a colorless substrate into a colored insoluble product that can be detected by light microscopy, ALPS patients show very low percentage of T cells that undergo apoptosis

**Case:** 18 mo. old girl, at check up presented with splenomegaly and lymphadenopathy, no unusual infections and seemed to be growing and developing normally, WBC count showed elevated lymphocytes, serum immunoglobulins all elevated, flow cytometry showed increased CD19 B cells, normal amounts of CD3 T cells but many were CD3alpha:beta double negative (for CD4 and CD8, abnormal), lymph node biopsy showed extensive enlargement of the follicles (hyperplasia) and increased immunoblasts and plasma cells in the paracortical area, no infectious agents were cultured from the node, no chromosomal abnormallity found on karyotyping and no evidence of oligoclonality of TCR (rules out malignancy), at adolescence lymph nodes decreased spontaneously, at 18 showed low platelet count due to an autoanitbody against platelets (diagnosis of idiopathic thrombocytopenic purpura- low platelets accompanied by red spotty skin discoloration due to local hemorrhages, treated with steroid dexamethasone), at 32 neutrophil county fell due to autoantibody against granulocytes, used TUNEL assay for apoptotic cells and determined that the percentage of T cells undergoing apoptosis was very low, DNA examination showed a single base transversion causing a premature termination codon in one of the alleles of the FAS gene, family history: paternal grandfather had splenomegaly and generalized lymphadenopathy and developed B cell lymphoma at 60, her father also has splenomegaly and lymphadenopathy

**Treatment:** based on immune suppression and surveillance of tumors (anti inflammatory steroid prednisone and immunsuppressant cyclosporin A), spleenectomy should be reserved for severe cases due to risk of infections by encapsulated bacteria

**Describe the role of Fas in inducing apoptosis in cells and that action is initiated by the binding of Fas to FasL (Fas ligand).**

Antigen specific lymphocytes are activated via their antigen receptors, undergo blast transformation, and undergo clonal expansion for up to 7-8 days to exponentially increase their numbers by cell division and predominate the population (in response to certain viruses, 50% or more of the CD8 cells at the peak of the response are for a specific single virus derived poptide:MHC1 complex). After clonal expansion, the lymphocytes undergo final differentiation into effector cells and remove the pathogen from the body, subsequently terminating the antigen stimulus.

After an infection has become overcome, the activated effector T cells undergo apoptosis (chromatin compaction, DNA fragmentation, cell shrinkage, internal degradation, shed membrane vesicles). One mechanism is the triggering of apoptosis via binding of Fas (TNF receptor family) with FasL (TNF family member of membrane associated cytokines), allowing for lymphocyte hemeostasis and cell death of the Fas-bearing cell via a caspase cascade. Binding of trimeric FasL to trimeric Fas brings the death domains in the Fas cytoplasmic tails together. Adaptor proteins such as FADD containing death domains bind to death domains of Fas, which interact with a death domain in protease caspase 8. Clustered caspase 8 transactivates and cleaves itself to release an active caspase domain that can cleave and activate caspase 3. Caspase 3 then activates caspase activatable DNase (CAD) present in all cells in inactive cytoplasmic form bound to the inhibitory protein I-CAD by I-CAD cleavage. CAD can then enter the nucleus where it cleaves DNA into 200 bp fragments. (Other mechanisms of apoptosis in immunity include the intrinsic mitochondria-dependent pathway in which cell damage, cytokine deprivation, and other mechanisms result in an increased release of cytochrome C contained in mitchondria and the activation of caspase 9).

**Explain that FasL and Fas are upregulated on lymphocytes during their clonal expansion in response to antigenic stimuli (i.e., during adaptive immune responses).**

Tc cells express FasL as a minor mechanism of cytotoxicity, wheras apoptosis in lymphocytes themselves induced through Fas seems to be an important mechanism of lymphocyte homeostasis.

**Recognize that a failure to make a productive Fas or FasL (or an active member of the caspase pathway leading to apoptotic cell death, such as caspase-10) leads to autoimmune lymphoproliferative syndrome (ALPS).**

**Recognize that normal B lymphocytes express Fas when they become activated, and that their expansion is controlled by interaction with FasL. Therefore, explain that B cells will accumulate in ALPS.**

**Explain that in ALPS there is an accumulation of B and T cells associated with lymph node enlargement, high blood immunoglobulin concentrations, and the production of autoantibodies (this condition is usually prevented via normal Fas:FasL action).**

**Recognize that the lack of normal elimination of B and T cells is also associated with development of lymphomas.**

**Viruses inhibit apoptosis so that the host cells in which they thrive don’t get eliminated by apoptosis induced by Tc cells. How is this accomplished?**

Vaccina expresses CrmA that inhibits caspases. HSV expresses Us5 and Us3 which inhibit caspases. EBV produced a protein that resembles Bcl-2 which prevents apoptosis and renders infected cells resistant to killing by Tc cells. (Bcl-2 binds to mitochondrial membranes and blocks the swelling so cytochrome C isn’t released and it can’t bind to Apaf-1, so no caspases are activated and CAD can’t enter the nucleus to cleave DNA)

**Case # 20: Hyper IgE Syndrome**

**Summary:** Naïve CD4 T cells differentiate into Th17 cells in the presence of IL6, IL21, and TGF beta. After their differentiation they produce cytokines IL17A, IL17F, IL21, and sometimes IL22. IL 17 induces epithelial and endothelial cells expressing the IL 17 R to produces chemokines (CXCL8) that attract neutrophils and monocytes. IL 22 induces keratinocytes to produce antimicrobial defensins (important for skin immunity). Characterized by the expression of the TF RORgT. Th17 cells have a role in the immune response (mucosal and epithelial surfaces) to extracelular bacteria and fungi and in autoimmune diseases.

IL6 has a key role in the differentiation of Th17 cells, in synergy with IL1 beta, IL21, and TGF beta. IL23 is important in amplifying the generation of Th17 cells and sustaining their survival. IL6, IL21, and IL23 all act on the same class of heterodimeric receptors (2 chains each associated with a JAK). Binding of dimeric ligand results in dimerization of the receptor chains and brings the JAKs close together so they can phosphorylate and activate each other. The activated JAKs phosphorylate tyrosines in the receptor tails. Members of the STAT family (STAT3, contains SH2 domains, inactive form in the cytoplasm), are recruited and bind to the tyrosine phosphorylated receptors and are then phsophroylated by JAKs. STAT proteins form a homodimer via the SH2 domains binding phsphotyrosine residues and translocate to the nucleus where they bind to and activate the transcription of a variety of genes important for adaptive immunity, such as retinoic acid related orphan receptor gT (RORgT) which is needed for the differentiation of activated CD4 T cells into Th17 cells.

Hyper IgE syndrome (Job’s syndrome) is a rare primary immunodeficiency characterized by a triad of symptoms including recurrent stph skin abscesses, pneumonia, and very high serum IgE levels. Recurrent staph skin abscesses that lack typical features of inflammation such as redness and warmth (cold abscesses). Association between cold abscesses, severe dermatitis, and high serum IgE. Patients are extremly susceptible to infections with S. aureus and Candida albicans and typically don’t mount strong inflammatory responses such as fever to infections.

Severe immunological and non-immunological manifestations. Eczematous rash in the neonatal period is usually the first clinical symptom. Staph skin abscesses occur in majority of patients and begin early in life. Recurren pneumonias (caused by s. aureus, strep pneumoniae, and h. influenzae) complicated by formation of pneumatoceles (thin walled air filled cysts in the lungs that can result from scarring) and dilation of the large airways. Systemic signs of illnes such as fever are often absent. Mucocutaneous candidiasis is common. Typical facial appearnce develops during childhood characterized by facial asymmetry, broad nose, deep set eyes, and prominent forehead. Musculoskeletal abnormalities are also common including scoliosis (survature of the spine), fractures occuring in setting of min trauma, and hyperextensibility of the joints. Most patients have delayed shedding of their primary teeth requiring surfical extraction. Increased incidence of malignancies, including lymphoma and leukemia.

Clinical diagnosis is based on clinical and lab findings. Scoring system was developed by NIH (scores over 40 likely to have HIES). Autosomal dominant and recessive forms known but in all cases the final common pathway is impaired Th17 differentiation.

Autosomal dominant form: more common, sporadic and familial inheritance, multisystemic disease, skeletal and dental anomalies as well as recurrent bacterial infections. Heterozygous mutations in the STAT3 gene (chrom 17) have been identified in majority of the patients, creating a dominant negative effect. Often within the DNA binding, Src homology (SH2), and transactivation domains of STAT3. See decrease in RORgT expression because STAT3 activity depends on intact function of both molecules of the dimer to induce expression of the TF. Cause of increased IgE is unclear.

Autosomal recessive: not typically associated with skeletal anomalies, bacterial infections, reucrrent and severe skin and MM infections with HSV and molluscum contagiosum virus, prone to develop squamous cell carcinomas. Mutations in genes for TYK2 and dedicator cytokinesis 8 (DOCK8).

Mouse models of STAT3 deficiency show that homozygous STAT3 KO are lethal (why no humans with homozygous STAT3 deficiency have been identified).

**Case:** 3 yr old boy with eczema, seborrheic dermatitis and neonatal acnes very early, both parents healthy, recurrent infections but was typically afebrile, skin infections with s. aureus, s. areus bursitis of the knee, pneumonia (complicated by pneumothroax), rhinosinusitis and chronic rhinorrhea that was usually yellow/green, recurrent otitis media that requires typanostomy tubes and lead to scarring, fractured arm on 2 occasions with minor trauma, chronic tympanic fluid drainage that grew s. aureus, prominent forehead and wide nasal bridge, low WBCs, high lymphocytes, high eosinophils, high IgM and very high IgE, sequencing of STAT3 gene showed heterozygous missense mutation in the DNA binding domain, CD4 T cells were stained for IL17A and assessed by flow cytometry, revealed significant decreased IL17A expression

**Treatment:** antibiotics, prophylactic bactrim, bone marrow transplant attempted in a patient but didn’t work (seems that defects apart from those in hematopoietic cell precursors are important in mediating the immunologic abnormalities, IFNg hasn’t impacted IgE levels much because the IgE is produced by activated B cells and plasma cells in which Th2 dependent class switching to IgE has already occurred (IgE synthesis by these cells is not subject to inhibition by IFNg which only counteracts Th2 cytokines induction of IgE switching in naïve B cells)

**Describe how cytokines regulate naïve CD4 T cells to become different functional sub-populations, namely T helper 1 (Th1), Th2, Th17 and regulatory T (Treg) cells.**

**Explain which cytokines are required to generate Th1 in comparison to Th2 or Th17 cells. Also, which cytokines are needed to generate Treg cells? How would you design culture conditions to obtain each of these populations?**

**Explain differences in the immune responses dominated by Th1, Th2 or Th17 cells. List the diseases that are associated with each of these populations.**

**Explain which cytokines are produced by Th1, Th2 and Th17 cells.**

**Provide information about transcription factors which are involved in regulating Th1, Th2 and Th17 as well as Treg cells. Explain how a mutation in the transcription factor may affect the immune response.**

**Explain the mechanism producing hyper IgE syndrome. What is the role of Th17 cells in this syndrome? What clinical symptoms are the most characteristic for this syndrome?**

**What information is needed to diagnose IgE syndrome? In addition to clinical symptoms, which additional diagnostics need to be performed to assure that this is hyper IgE syndrome?**

**What laboratory test indicates at hyper IgE syndrome? Most importantly, selective mutation in which gene is directly connected with hyper IgE syndrome?**

**Case 21: Ataxia Telangiectasia**

**Summary:** DNA is subject to damage during life by ionizing radiation, UV radiation, and ROS. Cause double strand breaks which are repaired by nonhomologous end joining (NHEJ) or homologous recombination repair. NHEJ joins DNA ends with little sequence homology and can occurs during Go, G1, and M phases. A complex of DNA repiar proteins bind the the broken DNA ends. The broken ends are processed by nucleases and DNA polymerases to remove or fill in ss overhands and the blut ends are then ligated. Usually leads to deletion or insertion of NTs at the site of the break. Homologous recombination repiar uses a sister chromatid as a template fore repairing the broken DNA. Because of the need for a sister chromatid, it only occurs during S and G2 phases after the chromosome has been replicated. Nucleases are recruited by a complex to transform the broken ends into longer ss overhands. One strand invades the DNA of the sister chromatid which is used as a template to repair the DNA break using sequence homology. Some of the same DNA repair proteins are used in both NHEJ and HR.

In many cases, repair begins with the recruitment of the MRN protein complex to the site of the break (MRE11:RAD50:NBS1), which facilitates the accumulation of additional proteins needed for repair. ATM (ataxis telangiectasia mutated, ser/thr kinase) is one of the first proteins recruited by the complex. ATM exists in the cytoplasm as an inactive dimer constitutively bound to a phosphatase that negatively regulates ATM kinase activity. When ATM binds to DS breaks, the phosphatase is released and ATM undergoes autophosphorylation and dissociates into active monomers.

After ATM activation, it phosphorylates a number of downstream proteins involved in DNA repair. One substrate involved in NHEJ is Artemis (nuclease that processes free DNA ends in preparation for ligation). Other substrates (such as the MRN complex) are important in HR. Activation of ATM substrates results in cell cycle arrest and DNA repair, or apoptosis if the DNA can’t be repaired. Redundancy in cell DNA repiar mechanisms permits some DNA to repair to occur even in the absence of ATM. However, cells that lack ATM don’t repair every break so ds breaks accumulate over time, manifesting as abnormal sensitivity of cells to ionizing radiation and the accumulation of chromosomal abnormalities.

In addition, some parts of the NHEJ pathway are used in the rejoining of gene segments to create signal and coding joints in VDJ recombination of Ig and TCR genes, as well as in class switiching (ds break produced in the Ig C regions). Both ATM dependent and independent pathways act in these processes.

Ataxia telangiectasia is an autosomal recessive disorder characterized by progressive cerebellar ataxia, neurodegeneration, oculocutaneous telangiectasias, primary immunodeficiency, and sensitivity to ionizing radiation. Caused by mutations in the ATM gene. Mutations are typically splice site or truncating mutations that lead to nonproduction of ATM proteins. Less commonly leakly splicing mutations or defects in the promoter result in low level expression of functional ATM protein resulting in a milder form. Most patients are compound heterozygotes for 2 different ATM mutations.

Gradual onset because there at ATM independent mechanisms of DNA repair with faulty repair of only a small fraction of dsDNA breaks, and the repair of ssDNA breaks doesn’t require ATM. Gradual onset is a result of slow accumultion of unrepaired dsDNA breaks.

Ataxia is the earliest sign of the condition and usually begins at 2-3 yrs. Although the cerbellum is affected first and most severely, the accumulation of damaged DNA in neuronal cells becomes more widespread and results in loss of gross and fine motor skills, dysphagia (difficulty swallowing), dysarthria (speech difficulty), abnormal eye movements, peripheral neuropathy and nerve dysfunction. The combination of ataxis with telangiectasis on the conjunctivae or pinnae is characteristics of AT. Another identifying feature is the elevation of AFP levels, usually more than 2 SD’s above normal.

Can have variety of defects in humoral and cellular immunity. ATM mutations may affect B cell and T cell development and function, leading to low numbers of B and T cells probably due to deleterious effects on VDJ recombination. Also impairs class switching so patients may have elecated IgM and decreased IgA, G, or E and poor response to polysaccharide Ags. Patients suffer frequent infections of sinuses and lungs (sinopulmonary infections) by bacteria such as s. pneumoniae and h. influenzae. Lymphocyte proliferation in response to motgens and Ags can vary from normal to impaired. Unlike patients with irradiation-sensitive SCID resulting from a deficiency of NHEJ components that are essential for VDJ recombination, patients with AT are not tpically at increased risk of opportunistic infections, indicating preservation of some immune function (especially T cell function).

Significant increased risk of leukemias and lymphomas. T and B cells routinely incur dsDNA breaks during Ag receptor formation and class switching. Translocations are most commonly found in these leukemias and lymphomas involving the TCR genes and Ig genes with oncogenes (growth advantage to abnormal cells resulting in clonal expansion). Also increased risk of solid organ cancers (breast cancer) caused by defective repair of ds breaks caused by ionizing radiation.

Differences in modifer genes can affect the clinical presentation of a monogenic disease. Have milder phenotype with delayed onset of symptoms, mild ataxia, no history of sinopulmonary infections (in mice model due to mutations in RAD50 gene can compensate for ATM deficiency).

Mutations in proteins that cooperate with ATM cause clinical phenotypes that overlap with AT. Hypomorphic mutations (decreased but not absent production/function of proteins) in MRE11 gene that encode part of the MRN comlplex results in a disease that looks like a mild form of AT with delayed onset and slower progression of symptoms. Mutations in another component of the MRN complex, nibrin, via the NBS1 gene causes Nijmegen breakage syndrome characterized by radiosensitivity, increased risk of leukemia and lymphoma, and immunodeficiency. Patients also have microcephaly, MR, and short stature but don’t have ataxia.

**Case:** 6 yr. old boy with history of recurrent ear infections and pneumonia, wobbly gait (ataxia) and dilated superficial blood vessels (telangiectasis) on conjunctivae and pinna, low total IgG, no IgG2 or 4, no IgA, and elevated IgM, positive Ab titer to one 1/14 peumococcal subtypes tested and none for h. influenzae even though he receieved both vaccines (poor Ab response), normal titer to tetanus, FACS showed low CD4 T cells, CD8 T cells, and B cells, proliferation of PBMCs to PHA and pokeweed mitogens and tetanus and diptheria Ag was normal, diagnosis made based on history of recurrent infections, ataxis, telangiectasis, hypogammaglobulinemia, and lymphopenia, measure blood alpha-fetoprotein (plasma protein produced by the liver that is elevated in ataxia telangiectasia, although not exclusively) and was shown to be high, assessed radiosensitivity of lymphoblastoid B cells by taking peripheral blood lymphocytes and transforming them with EBV, split them into 2 plates where 1 was irradiated and the other wasn’t, 10 days in culture and determine the survival fraction (number of surviving colonies in the irradiated plate with that of the nonirradiated plate) and found low survival fraction, sequencing of ATM gene showed compound heterozygote (2 different mutations with one in 1 ATM allele and another mutation in the other allele)

Could use western blotting to check for ATM gene because this is faster, but ATM is expressed at very low levels in peripheral blood lymphocytes and WB isn’t always reliable especially if the sample size is less then 10 mL. Best to diagnose based on combo of clinical, lab, and genetic testing. (No ATM detected by WB doesn’t necessarily mean they have AT)

**Treatment:** supportive, no targeted treatment, median age of death is 25 caused most often by cancer or progressive pulmonary disease resulting from recurrent infections, consider antibiotic prophylaxis in patients with repeated sinopulmonary infections, monitor pulmonary function, infusion of gamma globulin in patients with hypogammaglobulinemia or impaired specific antiobody production, minimize exposure to ionizing radiation in diagnostic studies

**Explain the importance of DNA repair in immune system development.**

**Explain how deficient DNA repair leads to an immunological phenotype.**

**List the diagnostic criteria for Ataxia Telangiectasia.**

**Describe why Ataxia Telangiectasia patients have an increased risk of leukemia and other cancers.**

**Radiosensitive T negative B negative NK positive SCID phenotype** (opportunistic infections, radiosensitivity, low T and B cells): caused by loss of function mutations in genes for Artemis, DNA ligase IV, Cernunnos, DNA PKcs (all essential for DNA repair in VDJ recombination and general DNA repair via NHEJ)

**Case 22: Warts, Hypogammaglobulinemia, infections, and myelokathexis syndrome (WHIM syndrome)**

**Summary:** Leukocytes generated in the bone marrow are released into the bloodstream where they populate peripheral lymphoid organs and patrol the periphery looking for pathogens. Effector leukcoytes circulate in the blood and can be specifically recruited to infection sites when required. Naïve lymphocytes continously recriculate from the bloodstream into peripheral lymphoid organs and back into the bloodstream. Leukocyte migration (homing) to lymphoid organs and infected tissues is controlled mainly via interactions of chemokines with their receptors on leukocytes. Chemokines produced at inflammation/infection sites attract leukocytes that express specific receptors for that chemokine, promoting their extravsasation. Chemokines are constitutively expressed by stromal cells in bone marrow and lymphoid organs that contribute to immune homeostatsis and maintain the structure of lymphoid tissues.

CXCL12 (stromal derived factor 1) is a key chemokine that directs homing of precursor and mature leukocytes to the bone marrow. Binds to CXCR4 receptor expressed by leukocytes. Levels of CXCR4 on leukocyte surface varies depending on their maturation and activation status, thereby modulating their responsiveness to CXCL12 and their tendency to home to the bone marrow.

CXCR4 (GCPR, 7 TMDs) is critical in guiding hematopoietic stem cells to the bone marrow during embryogenesis. In postnatal life, it helps to maintain hematopoietic stem cells in the bone marrow stem cell-niche. In myeloid lineage leukocytes, CXCR4 has a bimodal pattern of expression where higher levels are present on surfaces of immature myeloid progenitors and senescent neutrophils than on surfaces of mature leukocytes. CXCL12 is produced at high levels by bone marrow stromal cells, such that interaction of CXCL12 and CXCR4 promote the retention of immature myeloid progenitors in the bone marrow (permitting their maturation) and also favors the elimination of senescent neutrophils in the periphery by recruiting them to the bone marrow where they undergo apoptosis. (Myeloid cells show variable levels of CXCR2 expression which interacts with CXCL1 and 2 and has an important role in modulating circulation of myeloid cells). CXCR4 is also important for recirculation and homing of lymphoid lineage cells. Along with CXCR5 (receptor for CXCL19 and CXCL21) it modulates the migration of B cells withing the follicles and germinal centers of the lymph nodes. CXCR4 also has an important role in T lymphocytes and also is the co-receptor for HIV (facilitates infection of CD4 T cells). Mutations in CXCR4 leads to immunodeficiency resulting from deficiencies in leukocyte trafficking as result of defective interaction between chemokine and receptor.

WHIM caused by CXCR4 mutations. On chromosome 2. Very rare. Most mutations are heterozygous leadings to the premature truncation or disruption of the C terminal tail of the CXCR4 protein. Mutant protein is synthesized and expressed at the cell surface. May form homodimers or heterodimer complexes with normal CXCR4 protein. Dimers maintain ability to deliver intracellular activating signals but are resistant to beta arrestin dependent endocytosis. WHIM-causing CXCR4 mutants result in increased chemokine receptor signaling. Neutrophils and lymphocytes from patients showed increased chemotactic response to CXCL12. Small minority of patients are phenocopies with some having low levels of kinase G protein coupled receptor kinase 3 (GRK3, no mutations identified so far) which mediated CXCL12 dependent internalization and desnsitization of CXCR4 (acts as negative regulator of CXCR4 signaling).

Warts, hypogammaglobulinemia, infections, and myelokathexis. Patients may have incomplete phenotype. Warts may not become apparent until several years after birth. Peripheral leukopenia and marked reduction of absolute neutrophil count is almost invariably present. Hypercellularity in the bone marrow due to expansion of mature and apoptotic neutrophils. Myelokathexis indicates WBC retention. Neutropenia may be somewhat ameliorated (or even elevated) during acute infections when pro inflammatory signals outweight the CXCL12 mediated retention of neutrophils in the bone marrow (leave the bone marrow in response to strong inflammatory signals).

Recurrent bacterial infections are common, especially by h. influenzae, staph aureus, and s. pneumo. Infections often involve the respirtory tract, but deep seated abscesses can also occur. Hypogammaglobulinemia often present with decreased circulating B cells and reduction of switched memory CD27 IgD negative B cells. Patients can ount specific Ab responses after immunization, but the titer of specific Ab often wanes with time. Should use immunoglobulin replacement terhapy to reduce risk of bacterial infections. Also have reduction in naïve T cells and expansion of effector memory T cells shows restricted population.

Most patients develop persistant and treatment-resistant warts resulting from HPV infection. Usually hands but can be anywhere on body. Treatment with IV immunoglobulin doesn’t cause regression of the warts. Genital warts (condyloma acuminata) may predispose to epithelial cancer. Also see increased frequency of HSV, HHV8 Kapsoi Disease, EBV, and molluscum contagiosum infections. EBV can lead to lymphoma especially involving the skin. Defect in trafficking effector T and NK cells and APCs (DCs) in WHIM explains the abnormal vulnerability to viruses of the skin.

Higher incidence of congenital cardiac malformations. Consistant with role for CXCR4 in cardiac patterning.

**Case:** girl born with Tetralog of Fallot, severe neutropenia detected at age 2, recurrent bacterial penumonia with fever and cough, rales in R lower lobe and murmur at L sternal border, mild dyspnea, 10th centile for height and weight, no dysmorphic features, small tonsils, parents healthy, no other infectious diseases other than bacterial pneumonias, CXR confirmed lobar pneumonia and serum levels showed normal ANC but elevated CRP, week after tretment showed leukopenia, neutropenia, and lymphoenia, CRP had normalized, hypogammaglobulinemia (low IgG, IgA, and IgM), nonprotective antibody titeres to tetanus, h. influenzae, and strep pneumo despite immunizations, aberrant bone marrow aspirate showed very active myelopoiesis with myelokathexis (accumulation of senescent neutrophils characterized by pyknotic and hypersegmented nuclei), sequence of CXCR4 showed heterozygosity for R334X nonsense mutation, at 12 yrs begun to develop warts that are resistant to topical treatment and recur after curettage

Parents didn’t show symptoms. May be because her WHIM syndrome due to de novo mutation that arose in paternal or maternal germline. Or its possible that the disease wasn’t clinically evident in one of her parents despite the mutation.

**Treatment:** daily human recombinant granulocyte colony stimulating factor, regular admin of IV immunoglobulin, G-SCF and granulocyte macrophage colony stimulating factor, epinephrine, and glucocorticoids rapidly stimulate leukocyte release.

Receptor function of CXCR4 could be anagonized by competitive ligands such as AMD-100 (plerixafor) to interfere with CXCL12-CXCR4 interaction. Because CXC4 is important in retainign hematopoetic stem cells in the BM, this drug is sometimes used to help mobilze stem cells from the bone marrow to the periphery to facilitate collection of hematopoetic stem cells from blood (ex: for transplant).

**Explain what chemokines are and what roles they play in biology of leukocytes. How do chemokines regulate trafficking of leukocytes? The objective is to understand the concept of molecules regulating movement and destination of cells into tissues and organs.**

**Explain the role of CXCL12 (stromal-derived factor 1 [SDF-1]) that regulates the homing of leukocytes to organs such as bone marrow. This regulation depends on the expression of CXCL12 molecules on the surface of leukocytes. Explain how?**

**Which molecule is binding to CXCL12? Explain how these molecules function, and, in particular, how they regulate trafficking of leukocytes.**

**Characterize WHIM syndrome, and especially, describe the most characteristic symptoms associated with this disease. Why are hypogammaglobulinemia and warts symptoms in patients suffering from WHIM?**

**What genetic changes must be present to diagnose WHIM? In particular, which gene has selective mutation(s) indicating at this syndrome?**

Chronic neutropenia and hypogammaglobulinemia is also seen in CD40L or CD40 deficiency. X linked and autosomal recessive respectively, whereas WHIM is autosomal dominant. BM of CD40L/CD40 patients show arrest in myeloid differentiation, wehreas accumulation of mature neutrophils is seens in WHIM patients. Also, autoimmune neutropenia may be seen in CVID patients, and severe neutropneia can be transiently seen in patients with agammaglobulinemia during acute infections.

**Case 23: X-linked Hypohydrotic Ectodermal Dysplasia and Immunodeficiency**

**Summary:** Intracellular signaling molecules are vital for normal maturation of B and T cells. Although some molecules, such as CD40L, are specific for lymphocytes, others are found in many cell types. A crucial step in the activation of many different cells is the induction of the transcription of specific genes by NF kB TF’s. When not activated, NFkB and other family members reside in the cytoplasm where they are maintained in a complex with there inhibitor protein IkB. When the cell is activated via and exteral signal, a cascade of protein kinase activity is induced in the cytoplasm to cause assembly and activation of IkB Kinase (IKK) complex which consists of IKKa, IKKb, and NEMO (NFkB essential modifer)/IKKg. The IKK complex phosphorylates IkB which targets it for degradation by the ubiquitin-proteasome system to free NFkB. A nuclear localization signal is uncovered that allows NFkB to move into the nucleus where it binds to a particular NT sequence in the promoters of selected genes and initiates their transcription.

NFkB is activated by a variety of receptors, including those used in development, inflammation, and generation of immune response. Pro inflammatory cytokines TNFa and IL1 use NFkB. Also, TLRs on macrophages of the innate system recognize microbial components and other danger signals (LPS, PG, bacterial DNA CpG) to stimulate innative immunity. Also in B cells after stimulation via CD40 (adaptive).

Functional NFkB is measured by electrophoretic mobility shift assay. Detects its present in the nucleus. Cell nuclei are isolated from normal B cells via ultracentrifuation and their NFkB content is measured by assessing the capacity of nuclear extract to bind to a synthetic radiolabedl oligonucleotide DNA probe that binds to NFkB. The probe bound to NFkB is retarded in the gel as a result of the higher molecular weight of the protein probe complex. Although unstimulated B cells contain a baseline level of NFkB in their nuclei, ligation of CD40 by CD40L results in higher levels of NFkB in the nucleus.

X linked ectodermal dysplasia with immunodeficency is caused by hypomorphic mutations in NEMO genes that lead to production of a protein with impaired function. Results in immunodeficency because the end point of intracellular signaling pathways leading from a variety of receptors iimportant in activating immune response is NFkB activation and gene transcription. IN the absence of any compoenet of IKK (such as NEMO), IkB isn’t properly phosphorylated and it remains bound to NFkB preventing its translocation to the nucleus. Complete absence of NEMO (amorphic mutation) is lethal in the embryo.

Susceptible to opportunistic infections such as m. avium. One important defense against mycobacteria is activation of macrophages to synthesize cytokines such as IL18 and TNFa after recognition of microbes via TLRs. Cytokines amplify immune responses, activate macrophage intracellular killing and induce NO production which is important in destruction of the intracellular mycobacteria. In NEMO mutated patients, these cytokines aren’t made because TLR signal via NFkB activation is impaired. TNFa and IL18 also signal through NFkB pathway so their effect is severely reduced. This reduced the synthesis of IFNg by T cells which is normally induced by IL12 acting synergistically with IL18. Thus, NEMO deficiency also results in decreased IFNg porudction. Because IFNg also activates macrophage ability to kill intracellular bacteria, a lack of NEMO leads to inability of macrophages to kill mycobacteria that have been taken up phagocytosis and have become resitent in their endosomes. The persistently infected macrophages continue to activate T cells leading to granuloma formation. Patients have some similarities to patients with mutations in IL12 IL12R and IFNR mutations in their susceptibility to mycobacteria.

Suffer from viral infections such as CMV and herpes virus infections because killer activity of NK cells is deficient. Suggests that activation of NK cells via their invarient activating receptors in response to viruses is dependent on intact IKK and NFkB activation.

Interaction between CD40 on B cell and CD40L on T cell occurs but NFkB activation is impaired leading to diminished activation of mature naïve B cells resulting in impaired Ab synthesis and class switching (as assessed by a lack of upregulation of CD23 and CD54/ICAM-1 surface marks and decreased IgE synthesis measured by flow cytometry after CD40 ligation).

Patients have ectodermal dysplasia because a receptor that is required for ectodermal development depends on IKK function and NFkB activation to transmit signals to the nucleus. Ectodysplasin A receptor (TNFR superfamily) binds ectodysplasin A. Although a defect in these genes result in ED, there is no concomitant immunodeficiency because function of IKK complex is fine. Several hypomorphic mutation NEMO patients have been identified without ED. Alos, disctinct hypomorphic NEMO mutations have been charcerized that differentially impair innate and adaptive immune functions.

X linked, transmitted from mom to sons. Gene has 10 exons with most mutations causing ED in the 10th exon that carries a zinc finger domain that is a site of protein-protein interaction. Women who have a single copy of NEMO with a point mutation don’t have overt symptoms although they express both normal and mutant NEMO. Some may have missing teeth, mild abnormal patterns of hair or abnormal birthmarks. A major frameshift or deletion mutation in NEMO results in a condition called incontinentia pigmenti, characterized by dermal scarring and hyperpigmentation in heterozygotes. Doesn’t affect the immune system dbecause the normal NEMO genes allows appropriate immune system development and function. Immune cells of these women show nonrandom X chromosome inactivation with all of their immne cells containing the normal NEMO. Any male offspring of women with incontinentia pigmenti who receive the mutant NEMO die in utero.

**Case:** 9 mo old boy with strep meningitis but had been immunized (recurrent fever, nasal discharge, less interest in bottle, cried frequently, seizure, skin warm and reticular lacy pattern of BVs, rigid neck), WBC very high, 90% neutrophils, CSF was free flowing and cloudy with 12 RBCs and WBCs which were mostly neutrophils, elevated protein and decreased glucose, grew s. pneumo which expressed the p14 cell wall polysaccharide, low IgG, low normal IgM, normal absolute lymphocyte count and normal percentages of T and B cells, no specific antibodies to pneumo polysaccharides or tetanus toxoid although he had been immunized, dysplastic ectoderm (abnormality in growth of the structures produced from ectoderm, hair didn’t grow properly, teeth erupted at 21 months and were pointed and conical), lack of accrine sweat glands (hypohidrosis), ectodermal dysplasia with immunodeficiency suspected NEMO mutation, found point mutation that caused aa change at C term end of protein, remained well until 2 yrs when he developed a hyperpigmented lacy rash on back that revealed diffuse granulomatous inflammation and acid fast stain was positive for mycobacterium avium,

Residual serum levels of IgG and IgA because class switching can be induced by various TNF family members (CD40L) which are expressed on surace of activated DCs and engage B cell receptors. Activates NFkB in a NEMO independent manner by selective activtion of IKKa dimers by the kinase NIK resulting in processing of autoinhibited NFkB subunit p100 into active p52. This small amount of B cell activation may also explain the presence of lymph nodes in NEMO patients.

**Treatment:** immunoglobulin replacement threapy, antiboitcs

**Describe activation of NFB as being commonly found to be involved in the activation of many cells of the immune system involving both the innate and adaptive immune systems.**

**Explain that in its inactive form, NFB is complexed to IB and the complex is retained in the cytoplasm. Phosphorylation of IB leads to its degradation and release of NFB. The free NFB enters the nucleus to act as a transcription factor.**

**Explain that phosphorylation of IB is performed by a kinase activated via signal transduction systems linked to extracellular receptors (e.g. Toll-like, TNF-, and IL-1 receptors.**

**Explain that a major kinase involved in the phosphorylation of IB is represented by the IB kinase complex (IKK) and that one subunit of it is NEMO (an essential modifier of NFB)**

**Explain that mutations in the NEMO gene can lead to X-linked hypohydrotic ectodermal dysplasia with immunodeficiency and that this is consistent with NFB being needed for normal ectodermal differentiation.**

**Explain that complete mutations causing complete inactivity of NEMO result in failure to phosphorylate IB. These mutations are lethal in males and cause incontinentia pigmenti in heterozygotes (women), though the heterozygotes do not have a problem with immune function.**

**Describe the infections found in persons with reduced capacity to generate NFB and explain that these include those where T cells and activated macrophages are important in resistance (e.g. disseminated Mycobacterium avium), where opsonizing antibody from plasma cells is essential and is induced after activation and class switching of B cells (pyogenic bacteria), and where NK cells are helpful (several chronic viral infections).**

**Explain that the many different types of infection point to the importance of the NFB transcription factor in the activity of each of these types of cells.**

IkBa GOF mutation so it can’t be phosphorylated leads to more severe immunodeficiency

Possible that patients have NEMO deficiency despite normal coding sequence. Mutations in 5’ UTR may result in reduced mRNA and protein levels with normal coding sequence.